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Dynamics of cytomorphometric indicators in the epitheliocytes of the mucous membrane of the oral cavity of patients with generalized periodontitis under the influence of the treatment

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Abstract

There were examined 32 patients aged 22-44 years for some epigenetic mechanisms of the GP development; of them: 12 (by 6 men and women) were somatically and dentally healthy (group I, control) and 20 (by 10 men and women) patients with initial-I degree of the GP (group II). In cytological preparations of buccal epitheliocytes of the mucous membrane of oral cavity (MMOC) stained with aceto-orcein, 100 nucleus-containing epitheliocytes were examined using "Axioskop" microscope, Zeiss company (magnification: x 1000). Their cytomorphometric characteristics were studied: perimeter and area of the cell, perimeter and area of the nucleus, and the ratio of the area of the nucleus to the area of the cell before and after the complex therapy. All patients were performed initial periodontal therapy, local medicinal treatment using the solution "Phytodent" and ointment "Thiotriazoline", and endogenously "Thiotriazoline" was prescribed in tablets, as well as mineral complex "Calcemine". It was determined that in all patients with GP of the chronic course of initial-I degree compared with healthy ones, the cytomorphometric indices of MMOC of epithelial cells significantly reduce, namely: perimeter and area of cells and nuclei ($p \leq 0.05$; $p = 0.01$). Due to the use of the method of complex treatment developed by us, they significantly increased ($p_1 < 0.05$; $p_1 < 0.01$; $p_1 < 0.005$), practically reaching the data of healthy persons ($p > 0.05$). The gender peculiarities of the effectiveness of the complex treatment of the GP were in the more pronounced positive dynamics of morphometric parameters of epithelial cells and their nuclei in men, the increase of which was probable ($p_1 < 0.05$; $p_1 < 0.01$; $p_1 = 0.001$), compared with that in women; these indicators' growth in women was insignificant ($p_1 > 0.05$). The normalization of cells and nuclei parameters apparently occurred due to the pharmacotherapeutic effect of the used medicines, in particular, "Thiotriazoline", which positively affects the links of cellular metabolism that we've studied by improving the chromatin compaction in the karyoplasm.

Keywords: Generalized periodontitis, buccal epitheliocytes, cytomorphometry, complex treatment

Introduction

Generalized periodontitis (GP) – is a disease that is accompanied by gum inflammation and destruction of periodontal tissues and is the main cause of the teeth loss in the adult population. The significance of the microflora in the development of the disease is proven, but the severity of the inflammatory reaction is largely determined by the abilities of the macroorganism to withstand the action of pathogenic microflora on it [1]. In recent years, more and more works are devoted to the study of a number of factors that do not cause the disease in principle, but can complicate the course of the inflammatory process, contributing to the progression of the GP. These factors include the genetic status of a person.

Scientists of many countries determined that the changes of such clinical parameters as the depth of the periodontal pocket, the amount of epithelial attachment loss, and the magnitude of resorption of bone tissue are directly related to the genotype of the patient [2, 3]. It is known that all human cells contain the same genetic information that is read in the process of life selectively, since all genes never work. There is a selectivity of the inclusion or exclusion of certain genes while retaining the rest of the genetic information, and the selectivity of the read information is controlled by epigenetic mechanisms [4]. Determination of mechanisms of epigenetic regulation of genetic activity is necessary not only for understanding the foundations of ontogenesis, but also the causes of the emergence and development of various multifactorial diseases [5], in particular, the GP.

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Despite the significant progress in developing the new approaches to the GP treatment, their widespread use, ability to use multiple methods with the use of advanced technologies, the frequency and severity of this disease are not reduced. These circumstances determine the necessity to continue the search for new, more effective ways of studying the etiopathogenetic mechanisms of the GP to develop methods for primary and secondary prevention of the disease.

Materials and methods of research

There were examined 32 patients at the age of 22-44 years, among them: 12 (by 6 men and women) were somatically and dentally healthy persons (group I, control) and 20 (by 10 men and women) patients with the GP of the initial-I degree (group II).

The cells and nuclei of buccal epithelial cells of the mucous membrane of the oral cavity (MMOC) were studied. The material was taken with a sterile spatula not earlier than 2-3 hours after eating and brushing the teeth, with a fast, sliding movement along the middle line of the cheek. The smear was applied to a clean object carrier and was fixed with 96% alcohol during 5-10 minutes. Buccal smear in all women was taken in one period of the ovarian-menstrual cycle in a range of three days. In the smear the DNA was detected using the Feulgen reaction in the modification of L.Ye. Kovalchuk and co-authors [6]. In each preparation, with the help of the microscope "Axioskop", company Zeiss, (1000×magnification), 100 nucleus-containing epitheliocytes stained with aceto-orcein, were examined. The following cytomorphometric characteristics were studied: cell perimeter, cell area, perimeter of the nucleus, area of the nucleus, and the ratio of the nucleus area to the cell area was calculated (nuclear-cytoplasmic ratio).

All patients with the GP were performed a comprehensive treatment, which included initial periodontal therapy and medical treatment. Antiseptic rinses and oral baths with a solution "Phytodent" were prescribed (1 part of the medicine is diluted in two parts of water), the course – 7 days and applications on the gums and introduction into the periodontal pockets of the ointment "Thiotriazoline" during 25-30 minutes, the course – 7 days. Medications were prescribed: "Thiotriazoline" – 2 times a day (100 mg) during meals or immediately after it, the course – 20 days, and "Calcemine" – 1 tablet 2 times a day, the course – 15 days.

For statistical processing of the results the parametric methods of descriptive statistics (according to Student's t-criterion) were used.

Results of the research and their discussion

The dynamics of cytomorphometric indices in the epitheliocytes of MMOC before and immediately after the treatment, as well as in healthy individuals, was investigated in order to study the effect of the method used in our treatment of patients with the GP of the initial-I degree at the cellular level. In all patients, the perimeter and area of the buccal epithelial cells were decreased at 6.83% and 14.57%, respectively ($p < 0.05$), as shown in Table 1. After therapy, a significant increase in cell perimeter was found at 8.41% ($p_1 < 0.05$) and cell area at 17.37% ($p_1 = 0.01$) relative to the corresponding data before the treatment, exceeding those in healthy persons ($p > 0.05$).

Similar patterns were observed regarding the size parameters of the nuclei of epithelial cells: before treatment the perimeter and area of the nucleus were reduced at 9.57% ($p = 0.005$) and

15.53% ($p < 0.05$). As a result of integrated therapy, these indices increased at 7.51% ($p_1 < 0.005$) and 10.79% ($p_1 < 0.05$) respectively, and the difference with the data in the healthy ones became insignificant ($p > 0.05$).

The nuclear-cytoplasmic ratio in patients of the group II before and after the measures we've performed was close to the data in healthy ones, only the tendency towards its reduction as a result of treatment was revealed (at 3.70%).

The next stage of the work was the study of gender peculiarities of the dynamics of cytomorphometric indices of MMOC epitheliocytes in patients with the GP of the initial-I degree of different genders under the influence of complex treatment. It was determined that the perimeter and area of epithelial cells in patients who before the treatment were 9.03% and 21.13% ($p < 0.001$) lower than the data of group I (Table 2), after the treatment increased at 9.70% and 19.94% ($p_1 < 0.01$), respectively, almost reaching the control group data ($p > 0.05$). There was also a convincing increase of the perimeter and epithelial cells nuclei: if before our measures they were reduced at 16.12% ($p < 0.005$) and 29.63% ($p = 0.01$), then after – they increased at 9.08% ($p_1 = 0.001$) and 14.34% ($p_1 < 0.05$) respectively, with an approach to the data of healthy persons ($p > 0.05$).

The dynamics of the above-described morphometric parameters of the epithelial cells is consistent with the results of the determination of the nuclear-cytoplasmic ratio, which was reduced at 7.41% before the treatment, and after our measures there was a tendency to decrease this indicator at 3.85% more as compared with the data received before the treatment, and at 11.54% relative to the indicators of a group of healthy men.

The study of the dynamics of morphometric parameters of MMOC epitheliocytes in women with the GP (Table 3) determined a pattern similar to men: reduced before the treatment at 4.53% and 7.98% ($p > 0.05$), the perimeter and area of cells due to complex therapy increased at 7.54% and 15.64% ($p_1 > 0.05$).

Similar changes were observed regarding the perimeter and the area of the nuclei, which before the treatment in women were less than in healthy people at 2.98% and 2.08% ($p > 0.05$), and after the use of the developed by us medicinal complex increased at 5.94% and 6.68% ($p_1 > 0.05$), respectively.

In women of the group II, before the treatment, the nuclear-cytoplasmic ratio was somewhat elevated, and under its action of complex therapy there was a slight tendency to its decrease (at 7.41%), with the achievement of the ratio of the healthy persons' index.

Thus, a dynamic study of the morphometric indices of MMOC of the epitheliocytes in patients with GP showed that the complex treatment according to the developed by us method contributed to the complete normalization of the perimeter and area cell, as well as the perimeter of the nucleus and the approach to the data of the group of healthy individuals in the nucleus area index. Significant growth of these parameters, revealed during re-examination in patients of both genders, suggests that due to the therapy, the ability of decompaction of chromatin in the karyoplasm is optimized. However, the regulation of cytomorphological indices in men and women with GP was somewhat different. Gender peculiarities were that the indices of the epithelial cells and their nuclei in men changed significantly, and in women – less significantly. Normalization of cellular and nuclei parameters apparently occurred due to the

pharmacotherapeutic effect of the used medicines, in particular, “Thiotriazoline”, which positively affects the studied by us links of the cellular metabolism studied by us [7, 8], which was also found by other researchers [9, 10]. This is due to the fact that “Thiotriazoline” has a multifaceted action on the body as an antioxidant, membrane stabilizer and

immunomodulator. First of all, “Thiotriazoline” was used as a cardioprotective and hepatoprotective means, but nowadays it is widely used in gastroenterology, pediatrics, urology, surgery, ophthalmology, neurology, gynecology, and in the treatment of pathologies of different genesis [11, 12], in particular, in dentistry [13, 14, 15].

Table 1: Changes of the cytomorphometric indices of the epitheliocytes of the mucous membrane of the oral cavity in patients with the GP of the initial-I degree under the influence of complex treatment (M±m)

Indices	Group I Healthy (control) n=12	Group II patients with GP of initial-I degree	
		before the treatment, n=20	immediately after the treatment, n=20
perimeter of cell, μm	354.82±7.28	330.00±9.23 p=0.05	357.74±5.81 p>0.05 p ₁ <0.05
cell area, μm^2	8089.50±287.09	7051.23±357.78 p<0.05	8276.05±248.10 p>0.05 p ₁ =0.01
perimeter of nucleus, μm	58.95±1.31	54.06±0.10 p=0.01	58.12±0.69 p>0.05 p ₁ <0.005
nucleus area, μm^2	229.18±10.03	199.02±6.10 p<0.05	220.49±6.34 p>0.05 p ₁ <0.05
nuclear-cytoplasmic ratio	0.028	0.028	0.027

Note: The probability of difference of indices is indicated: p – to the value of indicators of healthy persons (group I); p₁ – to the value of the indicators before the treatment.

Table 2: Peculiarities of the dynamics of cytomorphometric indices of epitheliocytes of the mucous membrane of the oral cavity in men, healthy persons and patients with the GP of the initial-I degree, under the influence of complex therapy (M±m)

Indices	Group I healthy (control) n=6	Group II patients with GP of initial-I degree	
		before the treatment, n=10	immediately after the treatment, n=10
perimeter of cell, μm	363.15±3.38	332.53±5.55 p=0.005	364.77±8.36 p>0.05 p ₁ <0.01
cell area, μm^2	8558.05±287.09	7108.58±219.76 p<0.01	8525.69±364.39 p>0.05 p ₁ <0.01
perimeter of nucleus, μm	61.88±1.48	54.27±0.57 p<0.01	59.20±0.72 p>0.05 p ₁ =0.001
nucleus area, μm^2	251.12±11.85	195.28±4.71 p<0.05	223.28±9.69 p>0.05 p ₁ <0.05
nuclear-cytoplasmic ratio	0.029	0.027	0.026

Note: The probability of difference of indices is indicated: p – to the value of indicators of healthy persons (group I); p₁ – to the value of the indicators before the treatment.

Table 3: Peculiarities of the dynamics of cytomorphometric indices of epitheliocytes of the mucous membrane of the oral cavity in women, healthy persons and patients with the GP of the initial-I degree, under the influence of complex therapy (M±m)

Indices	Group I healthy (control) n=6	Group II patients with GP of initial-I degree	
		before the treatment, n=10	immediately after the treatment, n=10
perimeter of cell, μm	346.49±13.77	328.31±15.54 p>0.05	353.05±5.00 p>0.05 p ₁ >0.05
cell area, μm^2	7620.95±676.45	7012.99±603.30 p>0.05	8109.62±216.54 p>0.05 p ₁ >0.05
perimeter of nucleus, μm	56.03±0.29	53.84±2.01 p>0.05	57.04±0.97 p>0.05 p ₁ >0.05
nucleus area, μm^2	207.25±2.82	202.76±11.67 p>0.05	216.30±7.56 p>0.05 p ₁ >0.05
nuclear-cytoplasmic ratio	0.027	0.029	0.027

Note: The probability of difference of indices is indicated: p – to the value of indicators of healthy persons (group I); p₁ – to the value of the indicators before the treatment.

Conclusions

1. In all patients with the GP of chronic course of the initial-I degree of development compared with healthy ones the cytomorphometric indices of the MMOC epitheliocytes are significantly reduced, namely: perimeter and area of the cells and nuclei ($p \leq 0.05$; $p = 0.01$). Due to the use of the method of the complex treatment developed by us, they significantly increased ($p_1 < 0.05$; $p_1 < 0.01$; $p_1 < 0.005$), practically reaching the data of healthy persons ($p > 0.05$).
2. The gender peculiarities of the effectiveness of the complex treatment of the GP were in the more pronounced positive dynamics of morphometric parameters of epithelial cells and their nuclei in men, the increase of which was probable ($p_1 < 0.05$; $p_1 < 0.01$; $p_1 = 0.001$), compared with such in women, in which the growth of these indicators was insignificant ($p_1 > 0.05$).

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